



## Innovative Techniques for Large-scale Collection, Processing, and Storage of Eelgrass (*Zostera marina*) Seeds

by Robert J. Orth and Scott R. Marion

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**PROBLEM:** The use of eelgrass (*Zostera marina*) seeds is increasingly being recognized as a viable option for both small- and large-scale restoration projects. Although methods for hand-collecting, processing and storing eelgrass seeds have advanced to match the scale of collections, the number of seeds collected has limited the scale of restoration efforts, as well as the scale of ecologically relevant experiments. Recent experience with mechanized harvest of reproductive shoots has transcended the limitations of scale imposed upon divers working underwater during springtime with often severe weather limitations. Processing and storage methodologies and infrastructure previously scaled to hand collection now pose a bottleneck to the expansion of restoration and research to larger, previously unachievable scales.

**PURPOSE:** The purpose of this research was to develop methodologies for achieving the full potential for large-scale restoration presented by mechanized eelgrass seed harvesting. The specific goal in this project was to develop techniques and infrastructure to fully exploit potential mechanized seed harvesting capabilities, and to identify optimal conditions for storage and survival of large volumes of harvested seeds.

**BACKGROUND:** One of the goals of the Chesapeake Bay Program (Chesapeake Executive Council 2003, [http://www.chesapeakebay.net/info/pressreleases/ec/SAV\\_strategy.pdf](http://www.chesapeakebay.net/info/pressreleases/ec/SAV_strategy.pdf)) is to plant 1,000 acres of submerged aquatic vegetation (SAV) by 2008. In order to address this goal, methods must be developed for achieving the full potential for large-scale restoration as most previous seagrass restoration projects in Chesapeake Bay have occurred at relatively small spatial scales (<1 acre).

In developing the strategy, much attention was given to the need and process for carrying out large-scale restoration activities. Current SAV acreage in Chesapeake Bay is approximately 85,000 acres. The Chesapeake Bay Program has established a goal of 185,000 acres of SAV bay-wide by 2010. Even the ambitious goal of planting 1,000 acres by 2008 will provide only small progress toward the baywide, 185,000-acre goal. However, it is recognized that many regions within Chesapeake Bay have habitat suitable for SAV growth that are currently lacking vegetation, probably due to a lack of adequate seed or propagule sources. By identifying and strategically planting or seeding beds in these areas, it is expected that these beds would serve as a seed source to greatly accelerate natural revegetation on a much larger scale. The authors have achieved success in establishing large acre-size eelgrass plots from seeds in the Virginia coastal bays. This success demonstrates the utility of seeds in large-scale, seed-based restoration and the

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influence of recently established areas in providing seeds for natural expansion (Orth et al. 2006).<sup>1</sup>

To accelerate the pace of restoration, Virginia Institute of Marine Science (VIMS) and the Maryland Department of Natural Resources (MDNR) initiated a joint project in 2004 to explore the potential of a mechanized harvester (Figure 1) for collecting eelgrass reproductive shoots to provide the seed supply necessary for large-scale restoration. Approximately 10 million seeds were successfully collected and deployed, a trial of a new buoy-based seed distribution method was conducted (Pickerell et al. 2005, 2006),



Figure 1. Commercial mechanical harvester for large-scale collection of eelgrass reproductive shoots.

and damage to the source seagrass beds was verified to be minimal. The project demonstrated the clear potential of mechanical harvesting of eelgrass reproductive shoots for supplying large numbers of seeds. In comparison, approximately 2.5 million seeds were hand-collected in 2003. The project also illustrated a number of methodological bottlenecks to large-scale restoration. The mechanical harvester, originally designed for harvesting exotic species such as *Myriophyllum spicatum* (Eurasian watermilfoil) and *Hydrilla verticillata* (waterthyme), was contracted at significant cost, and its limited mobility presented logistical constraints to optimal siting of collections. Adapting to field weather conditions during the critical three- to four-week period in May when seeds are available for harvesting was difficult. Also, the experimental distribution of reproductive shoots in buoys immediately after collection presented numerous logistical challenges during the critical seed collection period, reinforcing the view that processing and storage of seeds will continue to be essential to achieving large-scale restoration.

This report addresses two aspects of large-scale, seed-based eelgrass restoration. The first section addresses techniques for maximizing seeds available for restoration, and the second section addresses an ongoing restoration project focused on seeding strategy.

## **DEVELOPING METHODOLOGY AND INFRASTRUCTURE TO MATCH THE POTENTIAL SCALE OF MECHANIZED SEED COLLECTION:**

**Optimizing Techniques for Mechanical Harvesting.** In 2004, flexibility in timing of collections was demonstrated to be key to optimizing the total seed harvest during the critical three-week window of opportunity. Among other logistical constraints, MDNR found a rapidly diminishing effort-return relationship as water temperatures increased, but the immobility of the contracted harvester made it difficult to use more optimal collection sites. Review of existing mechanical cutter designs identified components that could be easily adapted to a highly mobile, efficient mechanical harvesting system that achieves the simultaneous goals of maximizing

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<sup>1</sup> Unpublished data, Robert J. Orth, Department of Biological Sciences, Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Pt., VA.



Figure 2. Modified harvester designed to be operated from a small boat.



Figure 3. Harvester deployed from the side of a small vessel.



Figure 4. Pumping shoots directly into mesh bags (inside barrel).

collection capacity, efficiency, and seed yield per volume of grass collected, while minimizing impact on donor beds.

In 2005, VIMS designed and constructed a smaller, portable grass collection device consisting of a pair of horizontal, toothed cutting bars driven in opposition by an electric motor powered by a 12-volt battery (Figure 2). The cutting mechanism, a Lake Mower™ (Jensen Technologies Development Corporation, San Marcos, TX), is functionally similar to the larger commercial cutting apparatus used in 2004. The grass collection mechanism is scaled for use on a small boat that allowed easy deployment and relocation, and the height of the cutting bar is adjustable to target taller reproductive shoots while minimizing removal of vegetative leaves. The cutting mechanism is mounted on a benthic sled pulled alongside the boat by a beam, and is deployed by a davit and winch (Figure 3). Cut material is pumped from the collecting cage on the sled, via a Venturi nozzle attached to a gas-powered pump, directly into a mesh bag (Figure 4).

In 2004, eelgrass reproductive shoots were harvested from two locations in the Mobjack Bay–Browns Bay and Four Point Marsh. At Browns Bay, 1.68 million seeds were harvested from 3.7 acres of an 80-acre bed (Figure 5a). Similarly, at Four Point Marsh, 350,000 seeds were harvested from 2.2 acres of a 109-acre bed (Figure 6a). An aerial photograph taken on June 14 at both sites, approximately three weeks after harvesting, showed no tracks of the cutting machine (Figures 5b and 6b).

This harvesting device as constructed has limitations in cutting dense, long-leaved grass. While the cutting of plants is easily accomplished, the 3-in. diameter of the funnel and hose restricts the volume of material that



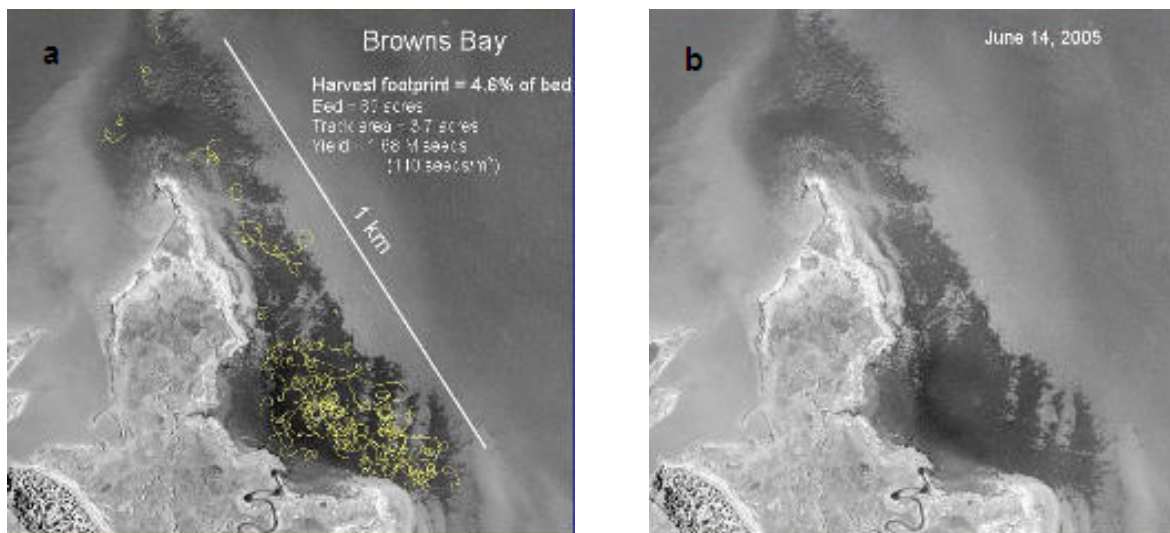


Figure 5. Aerial photographs of Browns Bay: (a) spiraling path of the cutting machine, (b) same photograph, revealing little visible evidence of impact along the harvester's track.

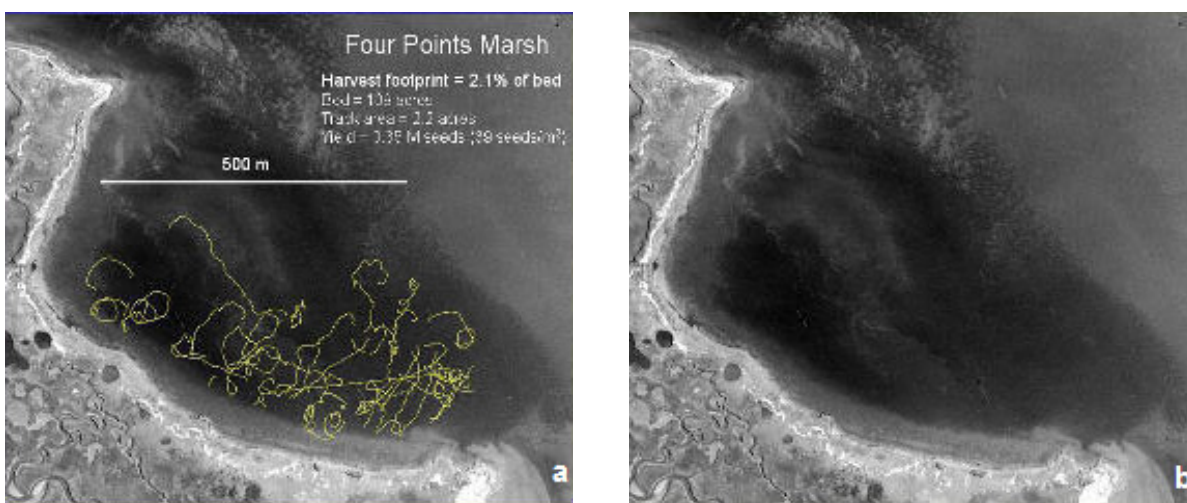


Figure 6. Aerial photograph of the grass bed at Four Points Marsh: (a) spiraling path of the cutting machine, (b) same photograph, revealing little evidence of impact along the harvester's track.

can pass quickly, causing clogs that necessitate removal and clearing of the funnel-hose connection. Increasing the hose diameter should allow more rapid collection from high-biomass beds. Also the precise targeting of small sections of the bed rich in reproductive shoots is made difficult by the position of the harvester alongside the boat, necessitating a spiraling boat path due to the off-center drag.

The harvester was reconfigured in 2007 to better target small areas of high-biomass beds identified during pre-season surveys as particularly rich in reproductive shoots. The sled was relocated to the front of a shallow-draft boat, and the collection apparatus was replaced with a net to passively catch the cut shoots (Figure 7). A system of ropes holds the net open at the

bottom just behind the cutting mechanism, and allows the net to be periodically pulled on board to empty the collected shoots. While more physically demanding than a pump-driven collection system, this configuration easily adapts to collecting in thick beds, and allows precise targeting of seed-rich zones.

With both designs, one person is required to operate the boat while a second individual is required to operate the cutting machine. A combination of the methods, pumping cut material through a large-bore hose with a powerful pump from a cutting sled located off the bow, may yield a more efficient, less labor-intensive process. By comparison, collecting reproductive shoots by hand requires many more hours in the water but yields a much richer density of seeds. If storage volume is a substantially limiting factor, hand-collecting may result in a greater total seed harvest under optimal collecting conditions in seed-rich beds.

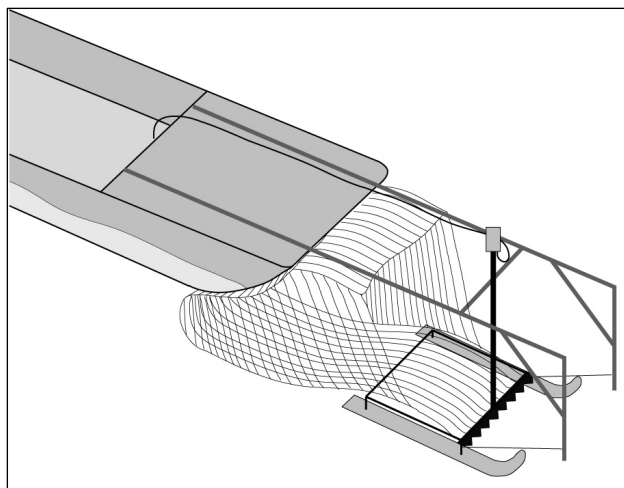


Figure 7. Modified harvester mounted to the bow of a boat.

### Expansion of Storage Capacity.

As the development of a mobile, versatile collection machine allows access to an unprecedented seed supply, the limiting factor will quickly become grass processing capacity. Because the mechanical harvester gathers a large volume of vegetative shoots along with the target reproductive shoots, processing the raw grass collections requires a much larger holding capacity than the current storage system allows. The



Figure 8. Outdoor eelgrass holding facility at VIMS.

capacity had previously been sufficient given the limitations of hand-collecting shoots and the high seed yield of those relatively pure reproductive shoot collections, but the dilution of collections with vegetative material requires more space. Figure 8 shows the previous and updated eelgrass seed processing facility at VIMS with plumbed fiberglass tanks. A plastic swimming pool was installed as a rapid, cost-effective, large-capacity solution for an unexpectedly large volume of material. All holding tanks are supplied with running seawater to allow a full exchange of water in approximately 2 hours, and air lines along the bottom (Figure 9) to actively aerate tank contents (Figure 10). Each tank holds between 250 and 350 gallons of loosely packed eelgrass shoots. The pool holds approximately four times the volume of the smaller tanks. All tanks are stirred daily until the seed separation process begins.

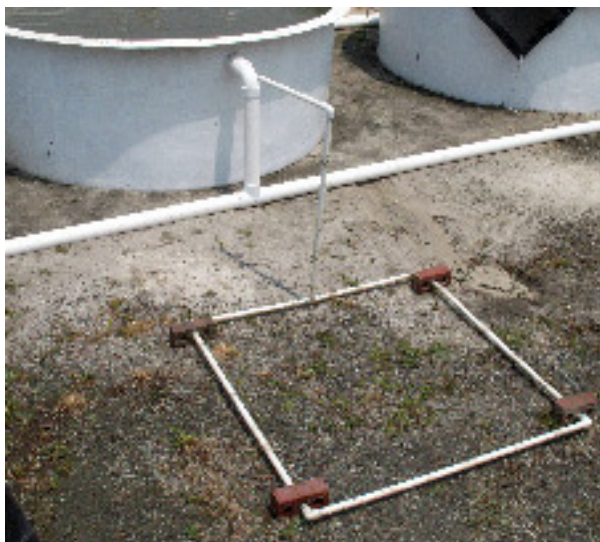


Figure 9. Square aeration mechanism placed on bottom of holding tanks.



Figure 10. Seed-bearing shoots are vigorously aerated in holding tanks.

**Enhancing Processing Efficiency.** The seed separation process for large volumes of material required a re-examination of traditional labor-intensive techniques. Traditional techniques require significant effort to remove large volumes of decomposing plant matter from the bottoms of tanks and sieving to separate seeds. To improve the process with a larger volume of material, a diaphragm pump capable of moving slurries of solids was used to streamline the material separation process. In addition, seeds were separated from vegetative matter using passive water flow by several methods (described below) to obtain a purer seed product that will help reduce risk of mortality-inducing hypoxic-anoxic events.

The process of separating seeds from decaying leaf material begins after all seeds have been released from the spathes. The duration of seed release is a function of the stage of development at which seeds were first harvested, the amount of material in the storage tanks, and water temperature. Results suggest that it can take from 4 to 6 weeks following collection to complete this process, which involves the following three stages.

**Stage I:** Removal of large reproductive shoot and leaf fragments.

*Manual method:*

- Stir the tank for 20-30 seconds to suspend grass fragments.
- Wait at least 10 seconds while good seeds fall back to the bottom. Most good seeds fall at roughly 4-6 cm/sec in 20-PSU water.
- Use coarse mesh screens (~2-in. mesh) to remove material that remains suspended in the top 30-50 cm of the tank.
- Repeat until little material appears on screens. Typically a tank that originally contained 250 gallons of loosely packed shoots can be screened in 1-2 hours.
- Removal from tanks: In round tanks with a removable central standpipe, create a vigorous circular current by walking rapidly around the tank, suspending the material remaining in the bottom. The resulting vortex eventually deposits the heaviest material (including good seeds)



near the center, with lighter grass fragments deposited closer to the tank walls. Then, by removing the standpipe and pushing the material closest to the center into the drain, a relatively clean batch of seeds can be collected on a 1-mm mesh screen or bag under the outlet pipe. The remaining material, containing a combination of seeds and grass fragments, can then be drained and further separated in Stage II.

In rectangular tanks or those without central drains, all material can be siphoned or pumped out of the tank with a diaphragm pump (which can directly pass grass and seeds without damage) for further separation in Stage II.

*Circular flow method:* By using the natural difference in fall velocity between good seeds and grass wrack, seeds (along with other materials of similar size/density such as barnacle shell fragments) can be passively separated. Using the 18-ft-diam circular swimming pool filled only with water, a slow, circular flow was created by angling the water inlet to one side. A diaphragm pump was then used to slowly introduce the grass/seed mixture from a holding tank (Figure 11). Water flow rates were calibrated such that almost all grass fragments settled to the bottom before circulating past the entry point. The best seeds fell several feet down-flow from the entry point, while most grass settled on the far side of the pool, with fine particles carried in eddies toward the center of the pool and fully around the perimeter (Figure 12). After all material has been deposited, the water level is lowered and seeds are siphoned off the bottom into 1-mm mesh holding bags. At greater distances from the entry point, the quality of the seeds declines (because of the reduced density of poor-quality seeds), so examination of extracted seeds is important for determining the point of diminishing returns.



Figure 11. Pumping seed/grass mixture into circular rotation for passive seed separation.



Figure 12. Grass wrack passively separated from good seeds.

**Stage II:** Separation of seeds from other small particles. Either Stage I method produces a mixture of good seeds, barnacle shells, and some small, heavy shoot fragments, requiring further separation to reduce the amount of organic material ultimately entering the seed storage phase.



*Flume method.* A simple flume was built by fiber-glassing a pyramid-shaped port onto an existing tank (Figure 13). A 2-in. layer of gridded plastic acts as a flow straightener at the upstream end of the tank, and the cone is filled with biological filter media (Bio Balls, Aquatic Ecosystems, Apopka, FL) that help create a relatively even flow field. A plexiglass window allows tests involving the calibration of flow rate versus fall velocity to be viewed. The bottom of the tank is 6 in. below the lowest point of water introduction, so once seeds settle near the bottom, they enter a region of slow-moving water and are not swept further down the flume. Handfuls of the grass/seed mixture are dropped into a 1-in. plastic mesh cage at the upstream end. The cage prevents clumps of grass from carrying seeds down the flume. Grass is agitated in the cage to facilitate separation. As the material falls through the mesh, good seeds fall rapidly to the bottom, while grass fragments are swept far enough down the flume that they become caught in the drain vortex and are carried out the drain. A large volume of material can gradually be added to the flume, after which seeds are siphoned off the bottom or pushed into the drain and collected on a 1-mm screen. Initial testing is critical to identify flow rates and water levels that prevent good seeds from being carried to the drain during separation.



Figure 13. Seed-separator flume.

*Trough method:* A similar concept can be applied in a long, shallow (2- to 3-in. deep) trough. The trough has a partition at the drain end that establishes the water depth, and water inflow at the opposite end is adjusted to create a slow, directional flow down the trough. A single batch of material (~ 2 L) is deposited at the top end all at once, and is very gently agitated by hand to help resuspend grass fragments. High-quality seeds remain close to the introduction point, while grass fragments and dead seeds are washed further down the trough. Each batch must be extracted separately; the good seeds are siphoned out, then the remnants are washed down the drain to clear the trough before the next batch of raw material is deposited.

**Stage III:** Final sieving. The seed mixture is sieved through a series of sieves (typically 2.0, 1.4, and 1.0 mm) to obtain a fairly clean batch of eelgrass seeds (Figure 14). Submerging the entire sieve set works better than pouring water onto the sieve. This is a fairly rapid process once the grass wrack has been removed. In some years, barnacle or slipper-shell fragments may be abundant in the final product. Shell fragments apparently do not influence seed survival as long as seeds are not subject to continuous mixing.



Figure 14. Final product of seed separation process.

**Optimizing Survival of Stored Seeds.** Past experience has shown dramatic variation in the survival rates of seeds between the stage of isolation from reproductive shoots and eventual distribution in the fall. Unknown factors have in certain cases resulted in large seed losses; past efforts have focused on preventing anoxic conditions and providing adequate water circulation, but factors leading to seed death were not well understood.

Avoiding infection, decomposition, and early seed death is critical to ensuring seed supply for large-scale distribution. The expansion of seed harvesting presents challenges with respect to the best methods for creating desirable tank conditions for volumes of seeds that are too large for conventional handling techniques.

In 2004, the importance of aeration and mixing on seed storage mortality was investigated, with the expectation that higher aeration would increase survival by eliminating anoxic conditions. Air treatments were compared with a treatment simulating seed burial immediately following release (buried under a 1-in. layer of sand), and a treatment testing the necessity of separating seeds from grass wrack (buried under a 1-in. layer of eelgrass detritus). Treatments are described in Table 1. Three replicate batches of 50,000 seeds each were established in outdoor flow-through seawater tanks. The experiment confirmed the importance of keeping seeds away from high-organic, low redox environments, as the buried seeds had poor survival (Figure 15). Unexpectedly, highly aerated seeds survived poorly relative to less-aerated treatments, and some seeds in the high air treatments germinated during the summer, a previously unobserved phenomenon.

<b>Table 1 2004 Seed Storage Experiment Treatments</b>			
<b>Treatment</b>	<b>Air</b>	<b>Mixing</b>	<b>Container</b>
High air	High flow	Constant	Cone bottom tank
Low air	Airstones	By hand, weekly	Flat-bottom tub
No air	None	None	Flat-bottom tub
Buried under sand	None	None	Flat-bottom tub
Buried under grass	None	None	Flat-bottom tub

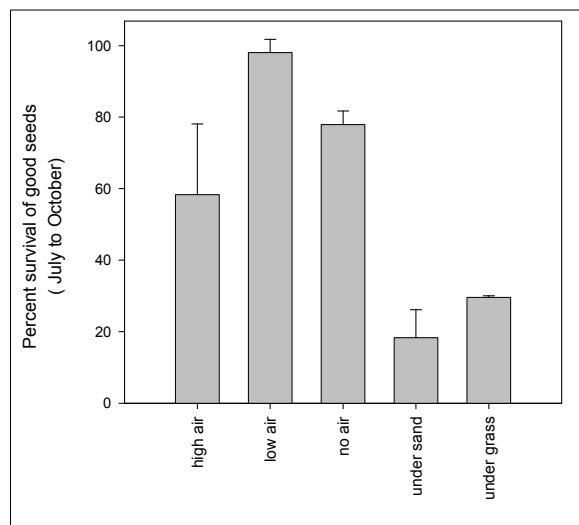


Figure 15. Results from 2004 tests of seed storage in relation to aeration (mean + standard error, n = 3).

A larger-scale replicated experiment was initiated in 2005 to further investigate the relationship between aeration, organic enrichment, and seed survival. Identifying that the buildup of fine particulates in flow-through seawater tanks was likely to exacerbate any problems with organic decomposition, seed survival was tested in outdoor raw and filtered seawater, and in indoor recirculating tanks. Treatments were similar to the three air treatments in the previous experiment, except that the high-air condition was achieved in a constantly circulating airlift chamber (Figure 16). Seeds stored in the indoor recirculating tank had the best overall survival, and aeration improved survival only in outdoor raw water, where it undoubtedly helped reduce accumulation of organic debris (Figure 17).

These results suggest that a primary consideration in storing seeds is minimizing the amount of organic material available to fuel microbial metabolism.



Figure 16. Circulating airlift systems used in 2005 seed storage tests.

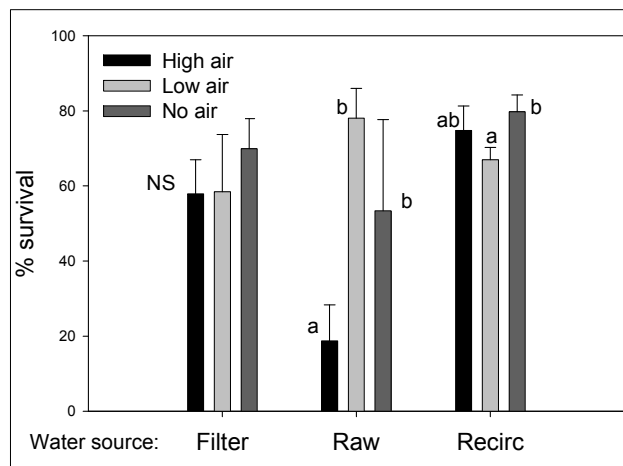


Figure 17. 2005 seed storage results. Distinct letters indicate significantly differing treatments within each water source ( $n = 4$ , SNK post-hoc multiple comparisons). (Recirc = Water was re-circulated, temperature controlled, sand filtered and UV-treated)

Since the indoor recirculating tanks were also temperature-controlled and constant-salinity (unlike the flow-through treatments), a third experiment was initiated in 2006 to assess the direct physiological impact of temperature and salinity on seed survival. Seeds were stored at salinities of 12, 20, and 30 PSU, representing the range of salinities at the potential restoration sites. Three replicates each (50,000 seeds) of each salinity treatment were placed in aquaria at three different temperature regimes: in a recirculating, temperature-controlled greenhouse tank (23-28 °C); in an air-conditioned lab (21-24 °C) (with aeration); and in a refrigerator (4 °C) (no air bubbling). Seeds were spread evenly across the bottom and not stirred during the course of the experiment. At all temperatures, low salinity was detrimental to seed survival, and high salinity supported optimal survival (Figure 18). Substantial seed germination during storage was observed in low-salinity treatments in the refrigerator (and to some extent in the lab), but since germination during storage precludes use of those seeds for restoration, refrigeration of seeds is not recommended. In the field, seeds germinate at temperatures below 15 °C. Recommendations for maximizing survival of eelgrass seeds during storage can be summarized as follows:

- Sieve seeds to remove as much organic material as possible
- Use recirculating water at moderate to high salinity (20-30 PSU)
- Keep temperature below 24 °C
- Avoid a thick layer of seeds (>3-5 cm) that might allow very low redox levels at the bottom
- Aerate the water, but leave the layer of seeds undisturbed
- If seeds are exposed to ambient light, shade the tank to reduce algal growth, and use a UV sterilizer to prevent growth of microorganisms.



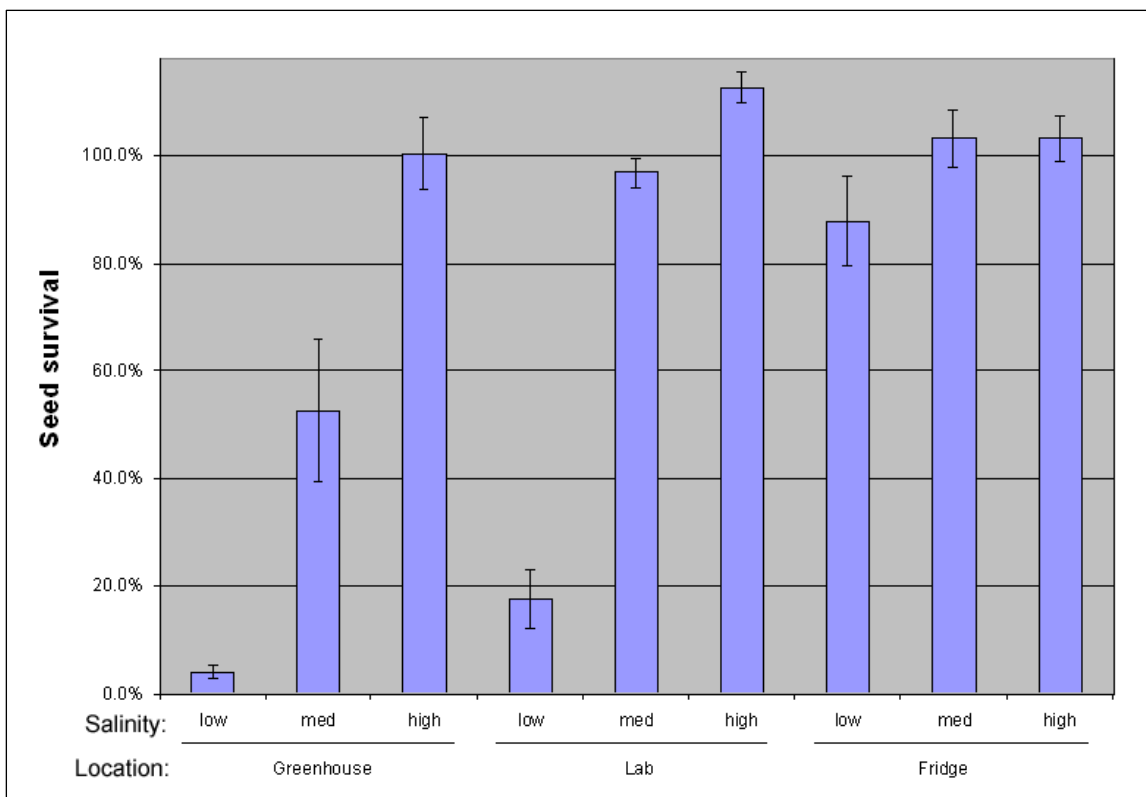


Figure 18. 2006 seed storage results (mean +/- standard error, n = 3). Survival over 100 percent reflects inaccuracies in volumetric seed measurement.

## EXPANDING RESTORATION SCALE PAST CRITICAL ECOLOGICAL THRESHOLDS:

The vast majority of past restoration attempts have been limited to plots under 10 m<sup>2</sup>. Among the larger scale projects, most have been initiated by seeds at extremely low densities (approximately 25-50 seeds/m<sup>2</sup>), with the objective of allowing subsequent growth and expansion to eventually fill in bare areas. Since a critical function of SAV beds is to self-modify their local environment by baffling wave and current energy, thereby enhancing deposition of fine particles and locally improving water quality, small-scale and low-density restoration attempts do not benefit from services that fully developed SAV beds provide. As a result, small, low-density plots may fail at a site where a fully developed bed would be able to persist, if only it could reach the density and scale where positive feedback mechanisms become functional. This restoration obstacle was identified as an important research item in the EPA Chesapeake Bay Program's recent "Strategy to Accelerate Protection and Restoration of Submerged Aquatic Vegetation in Chesapeake Bay." The level of seed collection facilitated by mechanized harvest will allow, for the first time, an assessment of high-density seeding at large scales.

In addition to scale, timing is a critical aspect of seed-based restoration success. High inter-year variability in conditions impacting seedling development, such as the timing of mid-winter storms that may uproot seedlings, or mid-summer temperatures during the seedlings' sensitive first season, may make repeated seeding efforts a key part of some restoration attempts. Restoration attempts are vulnerable in their early stages to disturbance events because the thin

canopy and sparse root mat do not provide the function of established grassbeds. In addition, since restored beds in the Chesapeake Bay do not produce reproductive shoots during their first year, there is no seed supply to allow a new bed to recover from damage. Experimental plots within the context of the larger restoration effort have been selected for repeated seeding in subsequent years until full coverage is reached and shoot density is similar to nearby natural beds, with the following goals:

1. Quickly develop a canopy thick enough to withstand disturbance events. Since broadcasting seeds more densely than  $1,250 \text{ m}^{-2}$  in a single year results in inefficient shoot competition, conducting seed broadcasts in subsequent years allows rapid development of thick cover while minimizing seed waste.
2. Increase the probability of recovery from natural disturbance events such as storm waves and bioturbation by cow-nose rays.
3. Simulate the natural mix of demographics within natural grassbeds by creating two year classes of plants. This combination may create a local population better suited to providing continuous supply of seedlings and vegetative growth in successive years.

In 2005, a 6-acre restoration plot in the lower York River was seeded (Figure 19). Transplants at this site, which historically supported dense eelgrass beds, have indicated adequate water quality for supporting eelgrass survival, but have not expanded significantly over the course of several years. The plot configuration (Figure 20) provides a large contiguous area that will ultimately enhance scale-related positive feedback benefits, but also allows comparison of seeding densities and repeated seeding effects. Two 1-acre subplots were seeded with 200,000 seeds each by hand-broadcasting while traversing the marked plots in a boat, and three adjacent plots were seeded with 100,000 seeds each. In 2006, the 200,000-seed plots were re-seeded with the same number of seeds, and a new 1-acre plot was seeded with 200,000 seeds. Each spring and fall, divers swim transects through the inshore and offshore portions of the plots to evaluate seedling germination and plot development. Table 2 shows plot performance to date. Plants are typically clustered, so the average density reported incorporates many barren areas in addition to high-density patches. Limited seed availability, combined with relatively low germination rates, has thus far kept the density of plants lower than targeted levels. Survival through the summer of 2006 appeared low, but continued survival and expansion of plants 18 months after broadcasting, even at the existing low density, is indicative of the potential for continued efforts at the site.



Figure 19. Site of the 2005-2006 large-scale seeding project.

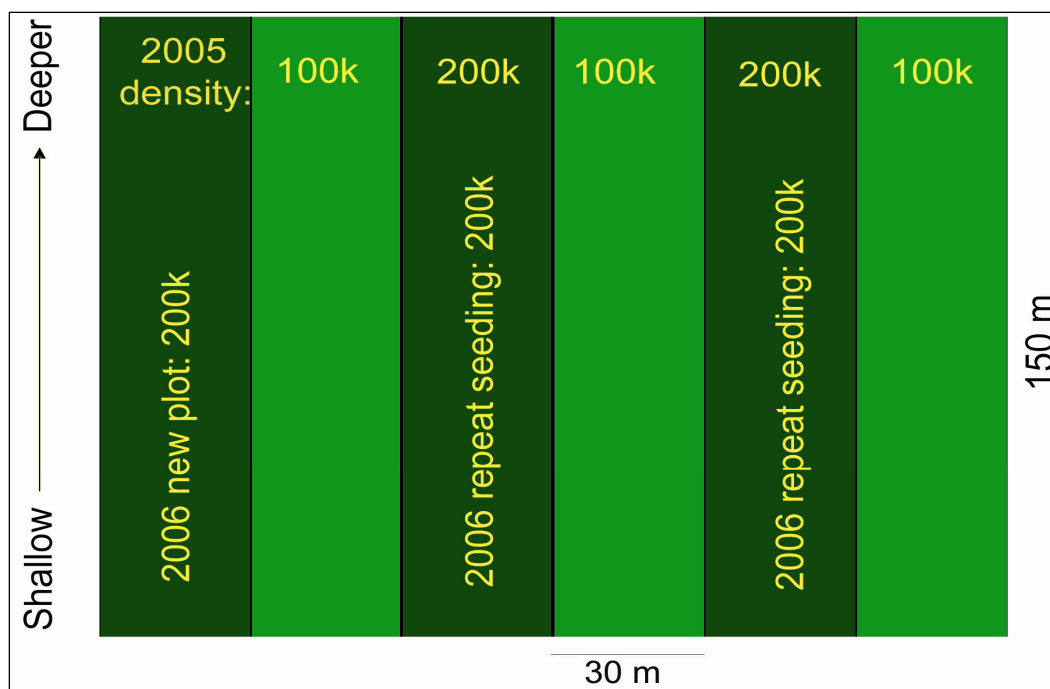


Figure 20. Plot layout for the repeated-seeding experiment.

**Table 2**

**Eelgrass Seedling Establishment and Survival in Plots Established in 2005 (plots 1-5) and 2006 (plot 6). Plots are physically arranged in the order presented in the table, forming one contiguous 6-acre plot. Plots 2 and 4 were re-seeded in fall 2006, along with Plot 6. Plots 1, 3, and 5 were not re-seeded**

Plot	2005 broadcast density (per acre)	Seedling establishment (Spring 2006 counts)			Summer survival (percent of Spring 2006 counts)			2006 broadcast density (per acre)	Spring 2007 density (plants / m <sup>2</sup> )			Spring 2007 survival (percent of Spring 2006 counts)		
		deep zone	shallow zone	plot mean	deep zone	shallow zone	plot mean		deep zone	shallow zone	plot mean	deep zone	shallow zone	plot mean
1	100,000	5.8%	5.7%	5.7%	5.2%	14.6%	9.9%	0	0.09	0.17	0.13	7.1%	13.7%	10.4%
2	200,000	3.6%	2.6%	2.6%	14.6%	17.4%	16.0%	200,000	0.73	1.02	0.87 *	not applicable		
3	100,000	4.4%	6.4%	6.4%	3.1%	6.9%	5.0%	0	0.10	0.28	0.19	10.1%	19.5%	14.8%
4	200,000	2.5%	4.2%	4.2%	21.5%	17.6%	19.5%	200,000	1.09	0.61	0.85 *	not applicable		
5	100,000	4.5%	3.4%	3.4%	7.5%	3.3%	5.4%	0	0.49	0.30	0.40	49.1%	39.8%	44.5%**
6	0							200,000	0.93	1.08	1.01 †	not applicable		
<b>Average:</b>		<b>4.2%</b>	<b>4.5%</b>	<b>4.5%</b>	<b>10.4%</b>	<b>12.0%</b>	<b>11.2%</b>							
* Combined density of second year adults and new seedlings. ** Plot 5 likely received some seeds from neighboring Plot 6 in 2006. † New seedlings only.														

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